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AGGLUTINATION OF STREPTOCOCCI, ESPECIALLY THOSE CULTIVATED FROM CASES OF SCARLATINA, BY HUMAN SERA.*

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EVER since it has been known that immune sera often agglutinate the bacteria concerned in their production, constant efforts have been made by investigators to determine the value of this reaction in the case of various bacteria, both as a means of diagnosis of specific diseases and as a method of differentiating bacteria which closely resemble each other. Streptococci have been included among the bacteria thus studied, and certain facts have been learned regarding the power of sera to cause their agglutination.

The first demonstration of the agglutination of streptococci by immune sera was furnished by van de Velde.[1] He studied the action of the sera of horses which were artificially immunized against streptococci, and found that an antistreptococcus serum possessed rather weak agglutinating power over the homologous bacterium, but not over other strains of streptococci. In 1897 Bordet[2] also determined that antistreptococcic serum possesses agglutinating properties, though in a feeble degree. He found that the agglutination of streptococci by preventive serum is usually distinct, but never great, and that a considerable quantity of serum is required to bring it about, at least one-third the volume of a fluid culture. R. Kraus,[3] writing upon agglutination, refers to observations by Widal in man which gave uncertain and variable results. His own investigations in this direction had not been satisfactory, and he concluded that little is to be expected from serum-diagnosis in streptococcus infections.

In 1902 Aronson[4] made very complete observations upon the agglutinating action of the sera of horses which had been immu-

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[1] *Arch. de Méd. expér.*, 1897, 9, 835.

[2] *Ann. de l'Inst. Pasteur*, 1897, 11, 177.

[3] *Wien. klin. Wchnschr.*, 1899, 12, 95.

[4] *Berl. klin. Wchnschr.*, 1902, 39, 979–982; 1006–1010.

nized against streptococci. The streptococci employed in the immunization and in the tests were rendered highly virulent by multiple passage through mice. He found that the serum from a horse treated with a certain streptococcus agglutinated other cultures of streptococci as well as the homologous one. His observations point to a close relationship between all streptococci. Agglutination was observed in dilutions of thirty times or less. There was no correspondence between agglutination and protective properties, and agglutination did not interfere with the life of the cocci.

Shortly after Aronson's publication, Moser and Pirquet[5] reported observations in connection with streptococci, and especially those coming from cases of scarlatina. Their findings were in some particulars exactly opposite to those of Aronson. From their experiments they arrived at the conclusion that streptococci from cases of scarlatina are different from those found in other human diseases. They tested the agglutinating power of human sera upon a streptococcus cultivated from the heart's blood of a fatal case of scarlatina in a child, with the following results:

1. Serum from cases of scarlatina agglutinates in slight dilutions in one-half the cases, the maximum dilution being 1:8.
2. Serum of other children agglutinates but rarely (three out of twenty-eight examinations), the maximum dilution being 1:4.
3. High agglutinating sera of horses injected subcutaneously in human patients always confers agglutinating properties upon human serum which are generally proportional to the quantity of serum injected and reach their highest point after twenty-four to thirty-six hours.

They found that normal horse serum agglutinated streptococci from various sources, but in moderate dilutions only, 1:4 to 1:64. The serum of horses immunized with streptococci cultivated from the heart's blood of fatal cases of scarlatina, and not passed through animals, agglutinated the same streptococci in very considerable dilutions (1:1,000 to 1:64,000). The same effects were produced upon streptococci cultivated from the heart's blood of cases of scarlatina, which were not used in producing the immunity (1:1,000 to 1:16,000). Streptococci from other diseases were agglutinated by the same sera at only a little higher dilutions than by normal horse serum (1:4 to 1:250), and the sera of horses immunized with these latter streptococci agglutinated streptococci from cases of scarlatina only in the same proportion as normal horse serum.

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[5] *Wien. klin. Wchnschr.*, 1902, 15, 1086.

Fritz Meyer[6] investigated the agglutination of streptococci by various antistreptococcic sera. He found that streptococci are agglutinated by their corresponding immune sera. By this means he undertakes absolutely to differentiate streptococci of anginas (scarlatinal, rheumatic, simple) from those of pyogenic infection. He finds gradual differences in the reaction of the anginal streptococci which speak against an identity among them.

Wlassjewski[7] studied the agglutinating effects of antistreptococcic serum (polyvalent), and of human serum from cases of puerperal fever, rheumatism and from healthy persons. The streptococci employed were from cases of scarlatina, puerperal fever, erysipelas, phlegmon, and dysentery. He found the polyvalent antistreptococcus serum agglutinated all the streptococci employed in the immunization, but some more strongly than others. The serum from puerperal cases agglutinated the puerperal streptococci at a dilution of 1:400 after twenty-four hours at 37° C. Serum from a case of rheumatism had no effect upon any of the cultures. He concludes that the reaction of agglutination is apparently specific, and may become a useful means of diagnosis and furnish a rational indication for the use of antistreptococcus serum in certain cases.

The agglutinating action of serum from cases of scarlatina upon scarlatinal streptococci has been studied also by Salge.[8] He found that streptococci from scarlatina were agglutinated by scarlatinal sera in dilutions up to 1:500. Agglutination did not occur with streptococci from other sources, nor with normal sera. Sera from other streptococcus diseases were not tested. He arrived at the conclusion that a distinct biologic relationship exists between scarlatinal streptococci and the serum of scarlatinal patients.

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[6] *Deutsche med. Wchnschr.*, 1902, 28, 751.

[7] *Centralb. f. Bakteriol.*, I. Abt., "Referate," 1903, 33, 464.

[8] *Ibid.*, 32, 643.

Stimulated by the reports of Moser and Pirquet, and Meyer, which appeared to indicate that varieties of streptococci could be recognized by differences in the agglutinating effects of anti-streptococcus sera upon them,· Aronson[9] undertook another series of exhaustive studies in order to learn whether his former results were reliable or not. In order that the conditions might correspond with those in the experiments of Moser and Meyer, horses were immunized with cultures of streptococci cultivated directly from man without passage through animals. The following cultures were employed for separate horses: seven various scarlatinal cultures, scarlatinal culture No. 8, puerperal sepsis No. 1, puerperal sepsis No. 2, articular rheumatism No. 1, articular rheumatism No. 2, otitis. Many other streptococci besides those employed in immunizing the horses were tested as to the agglutinating power of the various horses' sera upon them. Aronson found that streptococci cultivated directly from man are more readily agglutinated than those rendered virulent by animal passage. He also observed that cultures which inclined to produce a conglomerate growth were more easily agglutinated than those growing diffusely. The conclusion is reached from his agglutination tests that different groups of streptococci cannot be separated by this means. In his former work he had found that a serum prepared by immunizing with streptococci made virulent for mice agglutinated all such virulent cultures alike, but he now found that the serum prepared by immunizing with streptococci cultivated directly from man agglutinated in an entirely different way. The reaction with the latter serum was not obtained with all streptococci, nor with single groups, but was an essentially individual reaction. It was entirely indifferent whether the test culture originated from the same human disease or not. Not infrequently a readily agglutinated culture was agglutinated more by a serum prepared with a septic streptococcus than by one prepared with a scarlatinal streptococcus. The serum from the horse immunized with seven scarlatinal cultures agglutinated all the cultures employed. It agglutinated one scarlatinal streptococcus at a dilution of 1:20,000, another at 1:20, and a third not at all.

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[9] *Deutsche med. Wchnschr.*, 1903, 29, 439.

The serum from the horse immunized with scarlatinal culture No. 8 agglutinated the culture employed at 1:4,000, another scarlatinal culture at 1:50, while the serum prepared with a septic streptococcus agglutinated the latter at 1:100. The septic streptococus No. 1 was clumped by its homologous serum at 1:3,000, and not at all by the serum produced by septic streptococcus No. 2. Of the sera prepared from the two streptococci from articular rheumatism, each agglutinated its homologous culture, but not the other. Aronson concludes that in no case can a differentiation be made between streptococcus groups by the most careful agglutination tests.[10]

Early in the year 1903 the investigations about to be recorded were undertaken to determine whether streptococci from cases of scarlatina were constantly agglutinated by the sera of scarlatinal patients, and, if so, whether this was specific and might be of value in the diagnosis of doubtful cases. The technic employed has not differed materially from that of others who have used the macroscopic test. Some preliminary tests were undertaken by the microscopic method, but the results were usually so uncertain that it was abandoned. Aronson has insisted upon the necessity of using the macroscopic method for all non-motile bacteria in agglutination tests, and our results confirm his statements.

The cultures used were made in bouillon which was neutral to litmus and 1 per cent. acid to phenolphthalein. The growth in such bouillon was usually uniform. Each lot of bouillon before being used was tested with a culture known to be readily agglutinated, and with a serum previously found to agglutinate the culture; and if agglutination was not obtained, the entire lot of medium was discarded. Sometimes the reaction of the bouillon appeared to be at fault, a slight deviation in either direction being sufficient to interfere with the agglutination. If a culture in bouillon was found to be distinctly acid to litmus, it was not agglutinated by a serum which agglutinated the same streptococcus when grown in bouillon which remained neutral after twenty-four hours. If bouillon contained a small amount of sugar, the resulting production of acid interfered with agglutination. If alkali was added to such a culture in varying quantities, agglutination would occur near the neutral point (Table I). The effects of acid and alkali upon agglutination are shown in the accompanying tables (II and III). Cultures which grew conglomerately were not employed. It was found that cultures which at first grew conglomerately lost this property if grown for several generations in glucose bouillon with daily transplantations. Cultures which at first grew conglomerately were better agglutinated than those growing diffusely, as pointed out by Aronson.

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[10] Since this article went to the printer, two noteworthy communications bearing upon this subject have appeared. NEUFELD (*Zeit. f. Hyg. u. Infc.*, 1903, 44, 161) has studied immunity and agglutination in connection with streptococci. He found, like Aronson, that avirulent streptococci are more readily agglutinated than virulent ones. He also concludes that no specificity of streptococci isolated from scarlatina has been shown. MOSER AND PIRQUET (*Centralbl. f. Bakteriol.*, I, Abt., "Originale," 1903, 24, 560 and 714) have elaborated the microscopic method of studying agglutination of streptococci, and find it as reliable as the macroscopic. Their results do not show that the sera of horses immunized against streptococci from scarlatina produce agglutination of all streptococci cultivated from the blood of cases of scarlatina. They found the blood serum from cases of scarlatina to agglutinate a streptococcus from the blood of scarlatina, but only at low dilutions, not greater than occurred with sera from other diseases. Little value attaches to these observations, since only a single culture was employed, and the character of the control cases is not stated in detail.

TABLE I.

Showing that a Culture in Glucose Bouillon Becomes Agglutinable when the Acid in It is Neutralized.

Serum from Case of Scarlatina, No. 289	Streptococcus Culture No. 37, Grown in Plain Bouillon	Streptococcus Culture No, 37, Grown in 0.25 per Cent. Glucose Bouillon	$\frac{n}{20}$ Solution NaHO	Agglutination after Twenty-FourHours at Room Temperature
.....	1	..	.....	0
0.004	1	..	.....	+
.....	..	1	.....	0
0.004	..	1	.....	0
0.004	..	1	0.033	0
0.004	..	1	0.066	+
0.004	..	1	0.132	+
0.004	..	1	0.198	0
0.004	..	1	0.264	0
.....	..	1	0.033	0
.....	..	1	0.066	0
.....	..	1	0.132	0
.....	..	1	0.198	0
.....	..	1	0.264	0

The serum was obtained from blood drawn from one of the veins at the elbow by means of a Luer syringe, all precautions to

TABLE II.

Showing the Effects upon the Agglutination of the Additions of Alkali and Acid.

Serum from Scarlatina Case 292	Streptococcus Culture No. 37, Grown in Plain Bouillon	$\frac{n}{20}$ Solution NaHO	$\frac{n}{20}$ Solution H_2SO_4	Agglutination after Twenty-FourHours at Room Temperature
0.008	1	0.033	.....	0
0.008	1	0.016	.....	0
0.008	1	0.008	.....	0
0.008	1	0.004	.....	0
0.008	1	0.002	.....	+
0.008	1	0.001	.....	+
0.008	1	.....	.....	+
0.008	1	.....	0.001	+
0.008	1	.....	0.002	+
0.008	1	.....	0.004	+
0.008	1	.....	0.008	+
0.008	1	.....	0.016	+
0.008	1	.....	0.033	+
0.008	1	.....	0.066	0
0.008	1	.....	0.132	0

TABLE III.

Showing the Effects upon the Agglutination of a Streptococcus by a Normal Rabbit's Serum, Produced by the Addition of Alkali and Acid.

Normal Rabbit's Serum	Streptococcus Culture No. 45, Grown in Plain Bouillon	$\frac{n}{20}$ Solution NaHO	$\frac{n}{20}$ Solution Lactic Acid	Agglutination after Twenty-FourHours at Room Temperature
0.125	1	0.125	......	0
0.125	1	0.062	......	0
0.125	1	0.031	......	0
0.125	1	0.015	......	0
0.125	1	0.007	......	+
0.125	1	0.0035	......	+
0.125	1	......	......	+
0.125	1	......	0.0035	+
0.125	1	......	0.007	+
0.125	1	......	0.015	+
0.125	1	......	0.031	+
0.125	1	......	0.062	+
0.125	1	......	0.125	+
0.125	1	......	0.25	0
0.125	1	......	0.50	0

avoid contamination being observed. The serum was allowed to separate from the clot in the ice-box, and when used, a culture was made from the serum to insure its sterility. In making a series of tests, the serum was diluted with the same bouillon as that used for the culture. The dilutions of serum were of strengths of $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, etc. One part of these various strengths of serum was added to 30 parts of the twenty-four hours' bouillon culture. The mixtures of 1 part of serum to 8 parts of culture were made by means of drops from pipettes of uniform size. The mixtures of serum and culture were made in small test-tubes, each containing about 1 c.c. These were kept at room temperature for twenty to twenty-four hours, and then examined. When agglutination had taken place, the bacteria were collected at the bottom of the tube, leaving the upper part clear, and on shaking they rose in the fluid in flakes and granules which were not broken up by considerable agitation. When there was no agglutination, the bacteria which had settled to the bottom were uniformly distributed throughout the fluid on agitation. A control of the culture without any serum added to it was always kept in the same-sized tube and in the same quantity as the test mixtures; and if this control showed any conglomeration, the entire series was discarded. Microscopic examination of the clumps in the tubes exhibiting agglutination showed them to be made up of closely packed cocci. The chain arrangement was scarcely at all perceptible in these bunches. The rapidity with which agglutination appears is variable. It occurs more rapidly at incubator than at room temperature, often being complete in one hour, and usually in three to five hours. It was found that a corresponding agglutination took place after eighteen to twenty-four hours at room temperature, and this time was followed in our experiments. Room temperature has the advantage that little growth of streptococci occurs in twenty-four hours. If the mixtures are to be placed in the incubator, it would seem desirable to add formalin to the culture. We have found that 1 per cent. formalin has no effect upon the agglutination. In part of the sera tested the highest dilution of serum which produced agglutination was determined. In others the dilutions were carried to some point below the limit, lack of serum preventing further determinations.

In the tests here recorded the following cultures of streptococci were employed:

1. Eleven from cases of scarlatina: Nos. 11, 27, 28, and 36 from the tonsil, Nos. 37 and 47 from the blood during life, No. 39 from the lung after death, Nos. 40, 45, and 46 from the blood of heart after death, No. 44 from the pericardial exudate after death.
2. Two from phlegmons, Nos. 29 and 48.
3. One from the blood of a case of puerperal fever during life, No. 31.
4. One from the heart's valve in ulcerative endocarditis.
5. One from the peritoneal exudate in perforative peritonitis.
6. One from strangles in horses.
7. One from a rabbit after multiple passages.

Upon some of these cultures the agglutinating powers of each of the human sera from the following sources were tested:

a) Twenty-one cases of scarlatina. These represented all degrees of severity, and the blood was drawn from the second to the eighteenth day. Thirty specimens of sera were tested, twenty being obtained during the first week of the disease.

b) Seventeen cases of lobar pneumonia (nineteen sera). For these sera I am much indebted to Dr. E. C. Rosenow.

c) Four cases of erysipelas.

d) One case of endocarditis.

e) Three cases of measles.

f) Six cases of puerperal fever.

g) Two cases of typhoid fever.

h) Four normal individuals.

The accompanying table (IV) shows the agglutinating action observed with the sera from cases of scarlatina upon streptococci from cases of scarlatina. Cultures 28, 37, 45, 46, and 47 were agglutinated by each serum with which they were tested, the limit of dilutions at which the agglutination occurred varying from 1:30 to 1:4,000. Cultures 27, 39, 40, and 44 were not agglutinated by any serum with which they were tested. The sera which had no effect upon these latter cultures were always tested upon Nos. 28 and 37, and found to be active against them. It was not possible to demonstrate any connection between the dilution at which agglutination occurred, and the type of the case or the time in the disease when the serum was drawn.

The agglutinating effects of sera from lobar pneumonia upon cultures of streptococci from cases of scarlatina is shown in the accompanying table (V). It will be observed that the agglutinating action of the pneumonic sera was but little less than that of scarlatinal serum upon the same cultures.

TABLE IV

Showing the Agglutination of Streptococci from Cases of Scarlatina by the Sera from Cases of Scarlatina

Sera from Cases of *Scarlatina*		Streptococci from Cases of Scarlatina										
		11 Tonsil	27 Tonsil	28 Tonsil	36 Tonsil	37 Blood During Life, Case 270	39 Lung, Post-Mortem, Case 285	40 Blood, Post-Mortem, Case 285	44 Pericardial Exudate, Post-Mortem, Case 298	45 Blood, Post-Mortem, Case 300	46 Blood, Post-Mortem, Case 299	47 Blood During Life, Case 312
Number of sera tested (cases)		6(5)	2(2)	22(16)	3(3)	22(16)	5(4)	3(3)	2(2)	2(2)	5(5)	2(2)
Number of sera with which agglutination was obtained at various dilutions	1:8	2	..	..	..	..	..	..	..	..	..	..
	1:30	..	..	..	1	3	..	..	..	2	4	..
	1:60	1	..	3	..	1	..	..	..	..	1	1
	1:120	2	..	8	1	8	..	..	..	..	..	..
	1:240	..	..	6	..	3	..	..	..	..	..	1
	1:480	..	..	4	..	2	..	..	..	..	..	..
	1:1000	..	..	..	..	3	..	..	..	..	..	..
	1:2000	..	..	..	..	1	..	..	..	..	..	..
	1:4000	..	..	1	..	1	..	..	..	..	..	..
Number of sera with which the limit of agglutination was determined at various dilutions	1:8	2	..	..	..	..	..	..	..	..	..	..
	1:30	..	..	..	1	..	..	..	..	..	1	..
	1:60	1	..	2	..	1	..	..	..	..	1	1
	1:120	..	..	1	1	3	..	..	..	..	..	..
	1:240	..	..	3	..	..	..	..	..	..	..	1
	1:480	..	..	4	..	2	..	..	..	..	..	..
	1:1000	..	..	..	..	2	..	..	..	..	..	..
	1:2000	..	..	..	..	1	..	..	..	..	..	..
	1:4000	..	..	1	..	1	..	..	..	..	..	..
Number of sera with which no agglutination was obtained		1	2	0	1	0	5	3	2	0	0	0

Erysipelas serum possesses considerable agglutinating power over streptococci cultivated from cases of scarlatina, as shown in

Table VI. One erysipelas serum agglutinated streptococcus culture 37 at a dilution of 1:12,000, which was higher than was ever obtained with any other serum tested. The case from which this serum came was very severe and terminated fatally. The limit of dilution at which the sera from cases of puerperal fever will agglutinate these streptococci was not determined in most cases. The dilutions are, however, sufficiently high to indicate that agglutinating power is present in considerable degree (Table

TABLE V.

Showing the Agglutination of Streptococci from Cases of Scarlatina by Sera from Cases of Lobar Pneumonia.

Sera from Cases of *Lobar Pneumonia*		Streptococci from Cases of Scarlatina						
		11	27	28	36	37	39	40
		Tonsil	Tonsil	Tonsil	Tonsil	Blood During Life, Case 270	Lung, Post-Mortem, Case 285	Blood, Post-Mortem, Case 285
Number of sera tested (cases)		9(7)	3(3)	18(16)	1(1)	4(4)	1(1)	2(2)
Number of sera with which *agglutination* was obtained at various dilutions.	1:8	3	..	4	1	..	..	..
	1:30	..	..	2	..	..	..	..
	1:60	..	..	..	..	..	..	..
	1:120	3	..	4	..	3	..	..
	1:240	..	..	1	..	..	..	..
	1:480	..	..	1	..	..	..	..
	1:1000	..	..	2	..	..	..	..
	1:2000	..	..	..	..	1	..	..
Number of sera with which the *limit of agglutination* was determined at various dilutions.	1:8	1	..	3	1	..	..	..
	1:30	..	..	2	..	..	..	..
	1:60	..	..	..	..	..	..	..
	1:120	..	..	..	..	..	..	..
	1:240	..	..	..	..	..	..	..
	1:480	..	..	1	..	..	..	..
	1:1000	..	..	..	..	..	..	..
	1:2000	..	..	..	..	..	..	..
Number of sera with which *no agglutination* could be obtained.		3	3	4	0	0	1	2

VII). Sera from two cases of typhoid fever were tested upon scarlatinal streptococci. Both sera agglutinated Culture 47 at dilutions of 1:1,000, and Cultures 28, 37, 45, and 46 at dilutions of 1:30 to 1:120. Sera from three healthy persons were found

TABLE VI.

Sera Showing the Agglutination of Streptococci from Scarlatina by Sera from Cases of Erysipelas.

Sera from Four Cases of *Erysipelas*		Streptococci from Cases of Scarlatina						
		11 Tonsil	27 Tonsil	28 Tonsil	36 Tonsil	37 Blood, During Life, Case 270	39 Lung, Post-Mortem, Case 285	40 Blood, Post-Mortem, Case 285
Number of sera tested........		1	1	4	1	3	2	1
Number of sera with which *agglutination* was obtained at various dilutions.	1:8	1	..	..	..	..	..	..
	1:30	..	..	..	..	..	..	..
	1:120	..	..	1	..	1	..	..
	1:2000	..	..	2	..	1	..	..
	1:12000	..	..	..	..	1	..	..
Number of sera with which the *limit of agglutination* was determined at various dilutions.	1:8	1	..	..	..	..	..	..
	1:30	..	..	..	..	..	..	..
	1:2000	..	..	2	..	1	..	..
	1:12000	..	..	..	..	1	.	..
Number of sera with which *no agglutination* could be obtained.		0	1	1	1	0	2	1

to agglutinate part of the scarlatinal cultures at 1:4 to 1:15, but never higher. The sera from measles and a case of endocarditis had but feeble agglutinating power over this group of streptococci.

The streptococci from other sources than scarlatina were tested in a similar manner, and the results may be briefly summarized as follows: Culture 31, obtained during life from the blood of a woman with puerperal fever, was agglutinated by two scarlatinal sera at 1:30, by five at 1:8, and by two not at all; by one pneumonic serum at 1:120, by one at 1:30, by four at 1:8, and by eight not at all. Of the four erysipelas sera, one agglutinated it at 1:30, two at 1:8, and one not at all. Cultures 30 and 48, obtained from the pus of phlegmous, were agglutinated by various sera in a dilution of 1:8, but not higher. The other streptococci mentioned in the list were not agglutinated at all by most sera, and only with slight dilutions by a few.

TABLE VII.

Showing the Agglutination of Streptococci from Cases of Scarlatina by the Sera from Cases of Puerperal Fever.

Sera from Six Cases of *Puerperal Fever*		Streptococci from Cases of Scarlatina							
		11	28	36	37	44	45	46	47
		Tonsil	Tonsil	Tonsil	Blood During Life, Case 270	Pericardial Exudate, Post-Mortem, Case 298	Blood, Post-Mortem, Case 300	Blood, Post-Mortem, Case 299	Blood During Life, Case 312
Number of sera tested		1	4	1	5	2	4	2	1
Number of sera with which *agglutination* was obtained at various dilutions.	1:8	..	..	..	..	..	..	..	1
	1:30	1	1	..	1	..	2	..	..
	1:60	..	..	..	..	..	..	1	..
	1:120	..	1	1	1	..	1	1	..
	1:240	..	..	..	1	..	..	..	..
Number of sera with which the *limit of agglutination* was determined at various dilutions.	1:8	..	..	..	..	..	..	..	1
	1:30	..	..	..	..	..	..	..	..
	1:60	..	..	..	..	..	..	1	..
	1:120	..	..	..	..	..	1	..	..
Number of sera with which *no agglutination* could be obtained.		0	2	0	2	2	1	0	0

Streptococci which had been agglutinated by scarlatinal and erysipelas sera were found to be alive after twenty-four hours. It does not follow from this, however, that agglutination is dependent upon any vital action of the streptococci. Cultures killed with 1 per cent. of formalin were not altered in their agglutinable qualities, and cultures which had been kept for two weeks, and from which no growth could be obtained, were agglutinated as readily as live cultures of twenty-four hours' growth.

TABLE VIII

STREPTOCOCCUS CULTURE NO. 37 IN PLAIN BOUILLON		STREPTOCOCCUS CULTURE NO. 45 IN PLAIN BOUILLON		SERUM FROM PUERPERAL SEPSIS		SERUM FROM SCARLATINA NO. 299		SERUM FROM SCARLATINA NO. 300		AGGLUTINATION AFTER 24 HOURS AT ROOM TEMPERATURE
Unheated	Heated to 60–62° for 1 Hour	Unheated	Heated to 60–62° for 1 Hour	Unheated	Heated to 60–62° for 1 Hour	Unheated	Heated to 60–62° for 1 Hour	Unheated	Heated to 60–62° for 1 Hour	
+				+						+
+					+					0
+						+				+
+							+			0
+								+		++
+									+	+
	+			+						0
	+				+					0
	+					+				0
	+						+			0
	+							+		0
	+								+	0
		+		+						+
		+			+					0
		+				+				+
		+					+			0
		+						+		+
		+							+	0
			+	+						0
			+		+					0
			+			+				0
			+				+			0
			+					+		0
			+						+	0

In all the tests the serum was in a dilution of 1:30. In all except the last column of the table the + is used to denote the composition of the test mixtures. In the last column are shown the presence or absence of agglutination in mixtures of unheated and heated cultures with unheated and heated sera.

The experiments in the adjacent table (VIII) were made to determine the effects of heat upon the substances concerned in the agglutination reaction. It will be observed that cultures heated to 60–62° C. for one hour were no longer agglutinated.

Two of the sera lost their power of bringing about agglutination after being subjected to a temperature of 60–62° C. for one hour. One of the sera was still able to agglutinate one of the cultures, but in a reduced degree. These experiments show that streptococci possess a labile, functional substance which is readily destroyed by heat, as has been shown to be the case in typhoid bacilli. A second, more resisting substance, such as has been found in typhoid bacilli by Joos,[10] does not appear to be present. They also show that the substances in agglutinating serum which are essential for the production of visible agglutination are readily destroyed by heat. Posselt and Sagasser[11] have shown by specific absorption experiments that in the process of immunization with typhoid, coli, dysentery, and cholera bacteria the *Hauptagglutinin* is not only increased as the immunization progresses, but that also the *Nebenagglutinine* are increased; and while the dilution at which the injected organism is agglutinated is increased, there is also an increased agglutinating action upon the related bacteria. The tests upon the agglutinating effect of typhoid serum upon streptococci which have been referred to, while few in number, seem to indicate that in a similar manner substances which agglutinate streptococci are formed during typhoid fever. This is the more surprising as there is no intimate biologic relationship between the organisms concerned.

The results obtained by Aronson when he tested the agglutinating properties of immune sera produced by injecting streptococci grown directly from man correspond very closely to the results here recorded where human sera have been employed.

In looking for an explanation for the different results obtained by various observers, several factors suggest themselves as perhaps able to account for them in part at least. In Salge's experiments[12] conclusions are drawn from the results of tests with scarlatinal serum only, and without the essential controls. Aronson has pointed out that trikresol soon destroys the agglutinating properties of antistreptococcic sera, and so the value of many comparisons of the relative agglutinating power of different antistreptococcic sera is destroyed. As has been pointed out, slight variations in the composition and reaction of the culture medium may exert a marked effect upon the reaction.

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[10] *Centralbl. f. Bakteriol.*, I Abt., "Originale," 1903, 33, 762.

[11] *Wien. klin. Wchnschr.*, 1903, 17, 691.

[12] *Loc. cit.*

CONCLUSIONS.

1. Of streptococci cultivated from cases of scarlatina, some are agglutinated by almost all scarlatinal sera, but at dilutions varying from 1 : 60 to 1 : 4,000; others are agglutinated by the same sera with less constancy and at lower dilutions, and many are not agglutinated at all.

2. Streptococci cultivated from cases of scarlatina are agglutinated by sera from cases of lobar pneumonia and erysipelas at about the same dilutions as by scarlatinal sera, and in the case of erysipelas at even higher dilutions.

3. The same appears to be true of typhoid-fever serum, so far as limited tests indicate, and to almost the same extent of puerperal-fever serum.

4. The agglutination reaction between the streptococci cultivated from cases of scarlatina and the serum from cases of scarlet fever is in no way specific, and cannot be of any value as a means of diagnosis.

5. The effects here shown to be produced upon the agglutinating and agglutinable substances by heat, and the marked variations dependent upon slight alterations of reaction, serve to emphasize the importance of very exact methods in the study of agglutination phenomena in connection with streptococci, as well as with bacteria in general.